PB85-144129

Effect of Pentachlorophenol on the Growth and Mortality of Embryonic and Juvenile Steelhead Trout

(U.S.) Corvallis Environmental Research Lab., OR

1984

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

1. REPORT NO.	2.	3. PB85-144129
EPA-600/J-84-183		

4. TITLE AND SUBTITLE	5. REPORT DATE
EFFECT OF PENTACHLOROPHENOL ON THE GROWTH AND MORTALITY OF EMBRYONIC AND JUVENILE STEELHEAD TROUT (Journal Version)	1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Stephen E. Dominguez and Gary A Chapman	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS same as #12	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Corvallis, OR 97333	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/02

15. SUPPLEMENTARY NOTES

REF: ARCHIEVE OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY, 13:739-743, Nov. 1984

16. ABSTRACT

The toxicity of sodium pentachlorophenate to early life stages of steelhead trout (Salmo gairdneri) was investigated, using a flow-through exposure system. A 96-hr LC50 of 66 µg/L was derived for previously unexposed ten-week-old fry. A 72-day test, beginning 24 hr after fertilization, yielded a chronic toxicity threshold of 14 µg/L. These results, and the acute/chronic ratio of 4.7, compare reasonably well with values in the literature. Yolk sac edema and cranial malformations, which are commonly observed in chronic tests with technical grade pentachlorophenol, were rate in this test with purified (99%) material.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	Unclassified	6
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

Arch. Environ. Contam. Toxicol. 13, 739–743 (1984)

Archives of Environmental Contamination and Toxicology

EPA-600/J-84-183
JOURNAL ARTICLE

Effect of Pentachlorophenol on the Growth and Mortality of Embryonic and Juvenile Steelhead Trout

Stephen E. Dominguez and Gary A. Chapman

U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, 200 S.W. 35th Street, Corvallis, Oregon 97333

Abstract. The toxicity of sodium pentachlorophenate to early life stages of steelhead trout (*Salmo gairdneri*) was investigated, using a flow-through exposure system. A 96-hr LC50 of 66 µg/L was derived for previously unexposed ten-week-old fry. A 72-day test, beginning 24 hr after fertilization, yielded a chronic toxicity threshold of 14 µg/L. These results, and the acute/chronic ratio of 4.7, compare reasonably well with values in the literature. Yolk sac edema and cranial malformations, which are commonly observed in chronic tests with technical grade pentachlorophenol, were rare in this test with purified (99%) material.

Pentachlorophenol (PCP), a potent uncoupler of oxidative phosphorylation, has been widely used as a broad-spectrum biocide over the last several decades. While it is primarily used as a wood preservative, many other applications exist (Circelli 1978). Detrick (1977) reports annual worldwide usage to be approximately 90 million kg.

The water solubility of PCP, up to 4,000 mg/L as the sodium salt at pH 8 (Meyling and Pitchford 1966), is unusually high for an organic pesticide. Widespread environmental contamination that might be expected to result from such a circumstance has in fact been confirmed by a number of investigations, and has been reviewed by Ahlborg and Thunberg (1980).

A considerable amount of toxicity testing with a variety of organisms has been conducted to determine what effects such exposure might have on aquatic systems. While this data base is comparatively extensive, it does have some inadequacies. For example, it is known that PCP toxicity is influenced by temperature (Chapman *et al.* 1982; Hodson and Blunt 1981; Crandall and Goodnight 1959), pH (Kaila and Saarikoski 1977, Kobayashi and Kishino 1980; Chapman *et al.* 1982), and hypoxic stress (Chapman and Shumway 1978). Extensive inter-test disparities among these factors continue to pose interpretative difficulties. Moreover, there is a scarcity of acute toxicity data from flow-through tests in which pH, dissolved oxygen, and toxicant levels are less subject to alteration by metabolic activity. Also, contaminants associated with PCP may affect its toxicity (Nilsson *et al.* 1978; Ahlborg and Thunberg 1980; Cleveland *et al.* 1982). Yet, much of the available toxicity information was acquired with technical grades of PCP containing substantial amounts of impurities of unknown influence. Additional data from toxicity tests with purified PCP would provide a more consistent background against which other data would become more useful. Finally, while a number of fish growth studies have been conducted with PCP, few chronic or early life stage tests have been reported.

The purpose of the work described here was to investigate the toxicity of a purified preparation of PCP in acute and early life stage tests with steelhead trout (*Salmo gairdneri*), utilizing a flow-through exposure system.

Methods

Proportional dilutors were used, similar to those described by Mount and Brungs (1967). Each dilutor produced 24 L/hr of each of five different concentrations of PCP and one well-water control. These six flows were delivered separately to slate-bottomed 19-L glass aquaria modified to hold 13 L. The 90% water replacement time (Sprague 1969) was 75 min.

A purified (assay 99+%) preparation of PCP in the phenol

form, manufactured by Aldrich Chemical Co., Milwaukee, WI[1], was used in both tests. Toxicant concentrations quoted in regard to the tests are nominal values as the phenol.

Stock solutions were prepared by dissolving PCP in 0.5N or 0.05N NaOH, depending upon the PCP concentration needed; they were made in sufficient volume to last the length of the test and were stored in the dark under refrigeration. Well-water dilutions (0.4%) of these preparations, formulated in 35–50 L batches every 5–8 days, were used as working stocks in the Marriotte reservoirs of the dilutors. Opaque plastic covers over the reservoirs minimized photolysis of the PCP. Well-water dilutions of the working stock constituted the test waters, in which pH dictated predominance of the phenate form of PCP.

Illumination for the aquaria consisted of an 8-hr interval of dim light from two 20-watt incandescent bulbs, and a 16-hr bright period during which two 40-watt fluorescent tubes augmented the incandescents. Maximum light intensity at the water surface was 500 lux. Opaque plastic shrouds were used to screen the sides of the aquaria to eliminate behavioral interaction between fish in different aquaria.

Water temperature was maintained at 12 ± 1°C by means of a thermostatically controlled tempering tank upstream from the dilutors. Dissolved oxygen (mean 10.0, range 7.2 to 10.6 mg/L), total hardness (mean 29, range 25 to 36 mg/L as $CaCO_3$), total alkalinity (mean 26, range 23 to 31 mg/L as $CaCO_3$), and pH (mean 7.40, range 7.22 to 7.54) were measured weekly for the highest PCP concentration and one control solution. These factors did not differ significantly between test solutions.

For the chronic test, eggs were taken from several female steelhead at an Oregon Department of Fish and Wildlife (ODFW) egg-taking station on the North Fork Santiam River. After fertilization, the eggs were water hardened for one hr, transported to the laboratory, and deposited in a drawer-type vertical flow incubator. The next day, 24 ml of eggs (≈150 eggs) were transferred to each of 12 test incubators positioned individually within the aquaria of two dilutors. At this point, exposure to 10 different concentrations of PCP and a duplicated control was begun.

Each incubator (Stevens and Chapman 1983) consisted of a 22-cm length of 11-cm ID PVC pipe, sealed at one end (bottom) with a sheet of glass. A wooden lid limited light intensity within. Inside, glass beads 1.5, 1.0, and 0.4 cm in diameter were arranged in three layers, respectively, from the bottom up to a total column height of approximately 12 cm. Interstices of the top layer (0.4 cm beads) were sufficiently small to prevent passage of eggs or alevins down into the column, but large enough to prevent eggs from rolling in response to slight bumps or water currents and to provide an irregular surface for resting alevins.

Aquarium inflow from a dilutor mixing box was diverted into the incubator's external PVC standpipe and delivered to an injection point within the bead column one cm above the center of the bottom. From this point, water percolated up through the glass beads and exited through four screened ports located four cm below the top lip. The incubator's volume of about 1.1 L allowed a 90% turnover time of about six minutes.

Contents of the incubators were examined six days per week. Opaque white or fungused eggs were removed and cleared with Stockard's solution (Rugh 1952) to determine whether they had been fertilized. Unfertilized eggs (22 overall), and those damaged only by fungus from decomposing eggs nearby (20 overall), were not included in the test results.

[1] Mention of trade names or commercial products does not constitute endorsement or recommendation for use

After hatching, alevins were maintained in the incubators until they were ready to feed, at which time they were released into the aquaria. They were fed Oregon Moist Mash in an amount equivalent to 4% per day of control fish wet weight (about 15% per day based on dry weight of fish and food). The ration was adjusted weekly to account for growth and mortality. Fecal material and waste food were siphoned from the aquaria every second day.

Inter-treatment size comparisons were based on 20-fish samples from each aquarium. Sample fish were anesthetized with tricaine methane sulfonate, measured from tip of snout to end of caudal peduncle (variable fin erosion made use of total or fork length infeasible), blotted, and weighed on an analytical balance.

Live fish and eggs removed as samples were not returned to the test aquaria. There were accounted for by assuming that each sample would subsequently, over the interval to the next sampling or to the termination of the test, have suffered the same mortality as that experienced by the treatment population from which it was taken. Overall percent mortality (M) was calculated as follows:

$$M = 100\,[\,1 - (S_1 \cdot S_2 \ldots S_n)$$

where S_i is the proportion surviving over a particular sampling period.

One-way analysis of variance and the multiple comparison procedure of Dunnett (1964) were used to identify treatments yielding mean lengths or weights significantly different ($P = 0.05$) from control results. The threshold of chronic toxicity was estimated by calculating the geometric mean of the lowest effect concentration and the highest no-effect concentration as recommended by the U.S. Environmental Protection Agency (1980).

The 96-hr test was conducted in a third dilutor of the same design. Except for PCP exposure levels, test conditions were the same as in the chronic tests. The fish used were ten-week-old steelhead fry, averaging 264 mg in weight and 33 mm in length, which had been obtained as fertilized eggs at an ODFW hatchery on the North Fork Alsea River and reared at our laboratory.

At the start of the test, 20 fish which had been starved for 48 hr were placed in each of 12 aquaria by stratified random distribution of groups of five. The 12 aquaria accommodated duplicates of five PCP concentrations and a well water control. At the end of the test, control fish were weighed as described above and measured to fork length. The trimmed Spearman-Karber method (Hamilton *et al.* 1977, 1978) was used to calculate the LC50 and its 95% confidence interval.

Results

In the chronic test, abnormalities first became evident about the time the embryos' eye pigmentation became visible. At this point, about 17 days after fertilization, most embryos at 108 and 60 μg/L exhibited regions of interocular hemorrhage. Prehatch mortality (Table 1) was high at these concentrations, and the few alevins which hatched were smaller and less reactive than control alevins; all died within a week of hatch. Pre-hatch mortality was also significantly elevated at 34 μg/L, although much less so than in the higher concentrations. Nevertheless, post-hatch mortality of alevins at 34

Table 1. Chronic effects of pentachlorophenol (PCP) on steelhead trout mortality and growth

Nominal PCP concentration (μg/L)	% Mortality			Length on day 72 (mm)		Weight on day 72 (mg)	
	Pre-hatch	Post-hatch	Overall	Mean	Std. dev.	Mean	Std. dev.
0	4	9	13	30	2.0	335	7.5
1.7	3	12	15	29	2.7	311	9.1
2.6	6	12	17	32[a]	1.6	393[a]	8.1
3.9	7	8	14	30	2.7	309	8.2
5.8	6	2[a]	8	30	1.9	339	6.7
8.6[c]	—	—	—	—	—	—	—
11	5	9	14	29	2.5	285	8.2
19	8	32[a]	34[a]	28[a]	2.4	229[a]	6.4
34	14[a]	99[a]	99[a]	—[b]	—	—[b]	—
60	68[a]	100[a]	100[a]	—	—	—	—
108	99[a]	100[a]	100[a]	—	—	—	—

[a] Significantly different from controls at the 0.05 level
[b] The sole survivor at this concentration was not measured
[c] This concentration eliminated due to equipment malfunction

Table 2. Acute toxicity of pentachlorophenol (PCP) to steelhead trout fry

Nominal PCP concentration (μg/L)	Cumulative % mortality			
	25 hr	50 hr	72 hr	96 hr
0	0	0	0	0
34	0	0	2	5
50	0	0	12	24
74	2	32	46	58
111	83	100	100	100
166	100	100	100	100

μg/L was nearly 100%. Most of the hatch at 34 μg/L took place 25 or 26 days after fertilization, compared to 24 days for eggs at lower concentrations. At 19 μg/L, embryo mortality was not affected, but alevin mortality was still approximately three times that of the control; growth (both length and weight) was significantly reduced. Fish at 19 μg/L and higher concentrations generally exhibited fin erosion, mild cranial malformations, and lethargy in proportion to their level of exposure. Yolk sac edema, which had been observed in similar PCP studies in the past (Chapman 1969), was largely absent.

No adverse effects were seen at PCP concentrations of 11 μg/L or lower. Fry size was actually elevated at 2.6 μg/L, relative to the control. Growth stimulation at low concentrations has been encountered in other PCP studies (Hodson and Blunt 1981; Niimi and McFadden 1982). The phenomenon is not unique to PCP intoxication, and is generally attributed to overcompensation for a manageable degree of metabolic interference.

The acute test (Table 2) yielded a 96-hr LC50 of 66 ± 6 μg/L. Based on our calculated chronic toxicity threshold of 14 μg/L, the acute-chronic ratio was 4.7. This value compares well with ratios of 2.5 for *Daphnia magna*, 3.9 for *Pimephales promelas*, and 6.9 for *Cyprinodon variegatus* cited by EPA (1980) and based on studies by Adema (1978), Holcombe *et al.* (1982), and Parrish *et al.* (1978), respectively.

Discussion

Two studies similar to this one, in which rainbow trout were exposed to PCP from shortly after fertilization through an early juvenile stage, have been reported. Chapman (1969) found that 40 μg/L of a technial (90%) grade of NaPCP failed to elevate steelhead embryo mortality. It did, however, cause 100% mortality among the subsequent alevins. At 20 μg/L, alevin mortality was unaffected, but the dry weight of alevins was reduced nine percent relative to controls. These findings compare well with our thresholds of 34 μg/L for embryo mortality and 19 μg/L for juvenile mortality and growth.

In a study of temperature effects on chronic PCP toxicity, Hodson and Blunt (1981), using an analytical grade NaPCP, saw little or no induced mortality among rainbow embryos at concentrations up to 82 μg/L. Significant alevin and fry mortality occurred at 73 μg/L but not at 21 μg/L. Mean wet weight of fry was depressed by 25 μg/L PCP at 20°C, but was unaffected by 21 μg/L at 10°C. In general, effect concentrations reported by Hodson and Blunt are somewhat higher than ours, possibly due to differences in test conditions or toxicant purity.

Most estimates of the PCP 96-hr LC50 for

rainbow trout have been based on static tests. Seven such determinations have been reported using dilution water similar to the present study in terms of hardness (means of 5–35 mg/L as $CaCO_3$) and pH (6.9–7.1). These LC50s ranged from 50 to 100 μg/L (Davis and Hoos 1975; Bentley *et al.* 1975; Vigers and Maynard 1977), comparing favorably to our steelhead flow-through LC50 of 66 μg/L. Fogels and Sprague (1977) have reported a flow-through LC50 of 230 μg/L. However, this test was conducted in water having a hardness of 350–375 mg/L and a pH of 8.0–8.3.

Notwithstanding arguments that estimates of environmental levels of PCP may often be inflated by analytical pitfalls (Detrick 1977), the evidence of widespread aquatic contamination is compelling. Goto (1971) found PCP at 1–10 μg/L in rivers of southwest Japan, and lesser, but measurable, levels in most of the rest of the nation's rivers. Concentrations of 0.10–0.70 μg/L have been reported in the Willamette River, which drains much of western Oregon (Buhler *et al.* 1973). Concentrations up to 7.3 μg/L have been demonstrated in numerous fresh and marine waters of lower British Columbia, including Vancouver Island (Environment Canada 1979).

Ambient concentrations from this sampling of available reports fall below the threshold concentrations cited above for steelhead trout. However, those thresholds were derived with otherwise unstressed fish. Moderately unfavorable temperature (Hodson and Blunt 1981) or oxygen (Chapman and Shumway 1978) conditions contribute to sublethal effects and/or mortality at lower PCP concentrations.

Similarly, contaminants such as dibenzofurans, chlorinated dibenzo-*p*-dioxins, and other chlorophenols found in commercially used technical PCP may influence its toxicity. In 90 day chronic tests, Cleveland *et al.* (1982) found a composite of three technical PCP preparations to be capable of reducing the growth of fathead minnows at 13 μg/L, while a purified PCP had no impact below 85 μg/L. One of us (G. A. C., unpublished data) observed considerable incidence of yolk sac edema during earlier tests (Chapman 1969) in which steelhead alevins were exposed to technical grade PCP. Accumulation of fluid around the yolk sac sometimes approached 30% of the total wet weight of the alevin and was more severe when accompanied by lower dissolved oxygen levels. Yet, in the similar test reported here, in which purified PCP was used, edema was notably absent. The dioxins (hexa-, hepta-, and octachloro-) often found to contaminate technical PCP (Buser and Bosshardt 1976) are capable of producing edema in chicks (Flick *et al.* 1972), and tetrachlorodibenzo-*p*-dioxin has been shown to produce yolk sac edema in rainbow trout alevins (Helder 1981). It may be that one or more contaminant dioxins were responsible for the edema observed by Chapman (1969).

More information is needed on the chronic effects of PCP on most of the species of aquatic organisms which are commonly used in the setting of water quality criteria. The usefulness of such information would be enhanced if a better understanding was developed of how contaminants and other stresses affect responses of organisms to PCP.

References

Adema DMM (1978) *Daphnia magna* as a test animal in acute and chronic toxicity tests. Hydrobiologia 59:125–134

Ahlborg UG, Thunberg TM (1980) Chlorinated phenols: occurrence, toxicity, metabolism, and environmental impact. CRC Crit Rev Toxicol 7:1–35

Bentley RE, Heitmuller T, Sleight III BH, Parish, PR (1975) Acute toxicity of pentachlorophenol to bluegill (*Lepomis macrochirus*), rainbow trout (*Salmo gairdneri*), and pink shrimp (*Penaeus duorarum*). U.S. Environmental Protection Agency, Criteria Branch, Washington, DC Order number WA-6-99-1414-B

Buhler DR, Rasmusson ME, Nakaue HS (1973) Occurrence of hexachlorophene and pentachlorophenol in sewage and water. Environ Sci Technol 7:929–934

Buser H, Bosshardt HP (1976) Determination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in commercial pentachlorophenols by combined gas chromatography-mass spectrometry. J Assoc Anal Chem 59:562–569

Chapman GA (1969) Toxicity of pentachlorophenol to trout alevins. Ph.D. dissertation. Oregon State University, Corvallis, Oregon

Chapman GA, Shumway DL (1978) Effects of pentachlorophenate on survival and energy metabolism of embryonic and larval steelhead trout. In: Rao KR (ed) Pentachlorophenol. Plenum, New York, pp 285–299

Chapman PM, Farrell MA, Brinkhurst RD (1982) Relative tolerances of selected aquatic oligochaetes to combinations of pollutants and environmental factors. Aquat Toxicol 2:69–78

Cirelli DP (1978) Patterns of pentachlorophenol usage in the United States of America—an overview. In: Rao KR (ed) Pentachlorophenol. Plenum, New York, pp 13–18

Cleveland L, Buckler DR, Mayer FL, Branson DL (1982) Toxicity of three preparations of pentachlorophenol to fathead minnows—a comparative study. Environ Toxicol Chem 1:205–212

Crandall CA, Goodnight CJ (1959) The effect of various factors on the toxicity of sodium pentachlorophenate to fish. Limnol Oceanogr 4:53–56

Davis JC, Hoos RAW (1975) Use of sodium pentachlorophenate and dehydroabietic acid as reference toxicants for salmonid bioassays. J Fish Res Board Can 32:411–416

Detrick RS (1977) Pentachlorophenol: Possible sources of human exposure. For Prod J 27(6):13–16

Dunnett CW (1964) New tables for multiple comparisons with a control. Biometrics 20:482–491

Environment Canada (1979) Monitoring environmental contamination from chlorophenol contaminated wastes generated in the wood preservation industry. Regional Program Report 79-24, Environmental Protection Branch, Environmental Protection Service, Pacific and Yukon Region, West Vancouver, British Columbia, Canada

Flick DF, Firestone L, Higgenbotham GR (1972) Studies of the chick edema disease. 9. Response of chicks fed or singly administered synthetic edema-producing compounds. Poult Sci 51:2026–2034

Fogels A, Sprague JB (1977) Comparative short-term tolerance of zebrafish, flagfish, and rainbow trout to five poisons including potential reference toxicants. Water Res 11:811–817

Goto M (1971) Organochlorine compounds in the environment in Japan. In: Tahori AS (ed) International symposium on pesticide terminal residues. Pure and applied chemistry supplement. Butterworth's, London, pp 105–110

Hamilton MA, Russo RC, Thurston RV (1977, 1978) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–719. Correction 12:417

Helder T (1981) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of rainbow trout (*Salmo gairdneri*, Richardson). Toxicology 19:101–112

Hodson PV, Blunt BR (1981) Temperature-induced changes in pentachlorophenol chronic toxicity to early life stages of rainbow trout. Aquat Toxicol 1:113–127

Holcombe GW, Phipps GL, Fiandt JT (1982) Effects of phenol, 2,4-dimethylphenol, 2,4-dichlorophenol, and pentachlorophenol on embryo, larval, and early-juvenile fathead minnows (*Pimephales promelas*). Arch Environ Contam Toxicol 11:73–78

Kaila K, Saarikoski J (1977) Toxicity of pentachlorophenol and 2,3,6-trichlorophenol to the crayfish (*Astacus fluviatilis* L.). Environ Pollut 12:119–123

Kobayashi K, Kishino T (1980) Effect of pH on the toxicity and accumulation of pentachlorophenol in goldfish. Bull Japan Soc Sci Fish 46:167–170

Meyling AH, Pitchford RJ (1966) Physio-chemical properties of substances used as molluscacides. Bull World Health Org 34:141–145

Mount DI, Brungs WA (1967) A simplified dosing apparatus for fish toxicology studies. Water Res 1:21–29

Niimi AJ, McFadden CA (1982) Uptake of sodium pentachlorophenate (NaPCP) from water by rainbow trout (*Salmo gairdneri*) exposed to concentrations in the ng/L range. Bull Environ Contam Toxicol 28:11–19

Nilsson CA, Norström Å, Andersson K, Rappe C (1978) Impurities in commercial products related to pentachlorophenol: In: Rao KR (ed) Pentachlorophenol. Plenum, New York, pp 313–324

Parrish PR, Dyar EE, Enos JM, Wilson WG (1978) Chronic toxicity of chlordane, trifluralin, and pentachlorophenol to sheepshead minnows (*Cyprinodon variegatus*). U.S. Environmental Protection Agency Report EPA-600/3-78-010, Washington, DC

Rugh R (1952) Experimental embryology. Burgess Publishing Co, Minneapolis, Minnesota

Sprague JB (1969) Measurement of pollutant toxicity to fish: I. Bioassay methods for acute toxicity. Water Res 3:793–821

Stevens DG, Chapman GA (1984) Toxicity of trivalent chromium to early life stages of steelhead trout. Environ Toxicol Chem 3:125–133

U.S. Environmental Protection Agency (1980) Water quality criteria documents, availability. Federal Register 45(231): 79318-79345

Vigers GA, Maynard AW (1977) The residual oxygen bioassay: A rapid procedure to predict effluent toxicity to rainbow trout. Water Res 11:343–346

Manuscript received February 2, 1984 and in revised form May 4, 1984.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.